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RESEARCH ARTICLE

A case control study of association of vitamin D levels with uterine fibroids

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ABSTRACT

Objectives: To determine the association of low vitamin D levels and the occurrence of uterine fibroids. **Methods:** A case control study was conducted on 150 women between 20 to 55 years of age with at least one intrauterine fibroid of 2 cm^3 in volume or larger in transvaginal ultrasound (TVS) or transabdominal ultrasound (TAS) who were compared with 150 age matched controls. Fibroid volume and vitamin D levels were measured in the study patients. The relationship of vitamin D with the occurrence and volume of uterine fibroids was determined. $P < 0.05$ was considered as statistically significant. **Results:** The mean age of the study patients were 43.4 ± 5.28 years who presented with complaints and complications of prolonged/HMB (74.67%) followed by dysmenorrhea (44.00%), pelvic/abdominal pain (38.00%), anaemia required blood transfusion (31.33%), urinary complaints (20.67%), lump in abdomen (10.67%), dyspareunia (5.33%) and infertility (5.33%). Compared to controls, vitamin D levels were significantly less in cases (16.32 ± 8.58 vs. 31.98 ± 5.33, $p<0.0001$). A significant negative correlation was seen between vitamin D with fibroid volume ($r=-0.674$, $p<0.0001$). Low vitamin D was found to be an independent significant risk factor of presence of uterine fibroid (OR =89.763 for deficient vitamin D and 4.733 for insufficient vitamin D, $p<0.05$). **Conclusion:** In conclusion, low vitamin D carries significantly higher chances of developing big uterine fibroids. This opens up an interesting facet of dietary intake or supplementation of Vitamin D in prevention as well as treatment of uterine fibroid in women of child bearing age.

Keywords: Uterine fibroids, vitamin D, fibroid volume, supplementation.

The most frequent benign neoplastic threat in reproductive age women is uterine fibroids (UFs; leiomyoma), which occur in the myometrium. By the age of 50 years, 77% of women get these monoclonal tumours, whereas they manifest clinically in only 25%–50% of women. Even though these tumours are benign, they have significant morbidity involving anaemia, abnormal uterine bleeding, subfertility, pelvic pain, and obstetric complications. Also, these tumours are responsible for reproductive dysfunction and are considered to be the main sign for hysterectomy. UFs are a massive financial burden, calculated 34 billion dollars in the USA alone [1]. All these factors adversely affect a women's quality of life.

The effect of UF caused morbidities can be seen in all ethnicities, being commoner in African and Asian women. This disparity is thought to be associated with serum levels of vitamin D, like the risk of vitamin D deficiency is ten times higher in developing countries such as India and Africa [2].

For humans, vitamin D is considered to be one of the essential nutrients. As per some recent studies for uterine fibroids (UFs) development, vitamin D deficiency is required [3,4]. Bläuer et al [5] stated that both leiomyoma and primary myometrial cells showed an inhibited growth pattern corresponding to the concentration of vitamin D.

With the changing lifestyle, the deficiency of vitamin D is increasing. The reviewed literature shows that Indian

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studies are less which have determined the association of Vitamin D and uterine fibroids. The present study was conducted to estimate the prevalence of vitamin D deficiency among women presenting with uterine fibroids with an aim to determine the correlation of vitamin D levels with uterine fibroids in terms of its number and size.

Methods

A case control observational study was conducted at the gynaecology OPD of the department of Obstetrics and Gynaecology, Rabindranath Tagore Medical College and Pannadhay Rajkiya Mahila Chikitsalya, Udaipur, Rajasthan, over a period of one year from January 2019 – December 2019. The study was designed to determine the relationship between the plasma vitamin D levels and uterine fibroids in women between the age of 25-55 years attending gynaecology OPD.

Sample size: The study of Singh et al, [6] observed that mean vitamin D3 was significantly lower in cases than controls (10.81 ± 6.18 vs. 22.91 ± 16.18, $p < 0.0001$). Taking these values as reference, the minimum required sample size with 99% power of study and 1% level of significance is 50 patients in each study group. To reduce margin of error, total sample size taken is 300 (150 patients per group).

Inclusion criteria for cases:

1. Women between 25 to 55 years of age symptomatic for uterine fibroids attending gynaecology outpatient department in Pannadhay hospital.
2. Women with at least one intrauterine fibroid of $2cm^3$ in volume or larger on transabdominal ultrasound (TAS).
3. Women not fulfilling the exclusion criteria will be enrolled in the study.

Inclusion criteria for control:

1. Women between 25 – 55 years of age with diagnosis other than uterine fibroids attending gynaecology outpatient department in Pannadhay hospital.
2. Transabdominal ultrasound screening of fibroid free uterus in asymptomatic women was taken as control.

Exclusion criteria -

1. Non consenting women.
2. Women with ongoing pregnancy or pregnancy within 6 months prior to the start of the study.
3. Women currently lactating or history of lactation within 6 months prior to the start of the study.
4. Patients currently on calcium/ vitamin supplements or hormonal treatments and within six months prior to start of study.
5. Women with parathyroid dysfunction.
6. Women on antipsychotics, anticonvulsants, corticosteroids, anti-tubercular drugs (rifampicin), and HAART.
7. Women with chronic medical conditions like hypertension, chronic kidney disease, heart failure, angina, arrhythmias on calcium channel blockers medications.

Eligible subjects that met the inclusion criteria were included in the study group after appropriate consent was taken. They were subsequently matched for age, parity, and body mass index with women without uterine fibroids. This formed our control group.

On recruitment into study, a structured proforma was used to collect data regarding socio- demographic details, age at menarche, use of oral contraceptives, presenting complaints, obstetric and family history. Anthropometric measurements (weight and height) were taken. Thereafter transvaginal or transabdominal ultrasound as appropriate was performed on the subjects in the department of radiology in our institute. More commonly transabdominal ultrasound was done. Transvaginal ultrasound scan was performed on women with very small uterine fibroids not detectable via transabdominal ultrasound and also to confirm the absence or presence of atleast one uterine fibroid nodule of any size and the size of uterine fibroids measured. The ultrasound was performed irrespective of phase of the menstrual cycle.

Patients were adequately counselled before the procedure. The bladder was emptied for the transvaginal scan while transabdominal scan required a full bladder. For each case subject, total volume of all existing fibroids were measured in three perpendicular planes, with volume determined according to the prolate ellipse formula (a x b x c x 0.523), where ‘a’ is height, ‘b’ is width and ‘c’ is depth.

With the patient sitting in upright position, a tourniquet was applied for a few seconds (with minimal stasis) and 5ml of venous blood was collected from the antecubital vein by means of venopuncture into the vacutainer tubes containing lithium heparin from participants in the study and control group. Then samples were taken to the laboratory within 2 hours collection for separation, storage and analysis. The accepted biomarker of vitamin D status is circulating levels of 25 (OH) D. Serum levels of 25-Hydroxyvitamin D3 is the best indicator of vitamin D status because it reflects total vitamin D from sunlight exposure, dietary intake, and the conversion of vitamin D from adipose stores in the liver.

The method used for analysis was electrochem-iluminescence binding assay. It is intended for quantitative determination of total 25-hydroxyvitamin D in human serum and plasma on Elecsys and Cobas-e immunoassay analyzers.

The primary outcome measures were vitamin D deficiency among women with uterine fibroids and the secondary outcome measures were its correlation with fibroid volume.

Statistical analysis: The comparison of the age was compared between cases and controls using Independent t test (for two groups) and ANOVA was used for comparison of vitamin D (ng/mL) between age groups and body mass index. The comparison of the variables like area of residence, education, religion, occupation, age of menarche, parity, body mass index, family history and use of OCP between cases and controls and association of prolonged/ HMB, dysmenorrhea, pelvic/abdominal pain, urinary complaints and anaemia required blood transfusion with vitamin D (ng/mL) were analysed using Chi-Square test. Fisher's exact test was used for association of dyspareunia, lump in abdomen, RPL and infertility with vitamin D (ng/mL). Pearson correlation coefficient was used for correlation of vitamin D levels with fibroid volume in cases. Multivariate logistic regression was used to find out independent risk factors of uterine fibroid. The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 21.0. For statistical significance, p value of less than 0.05 was considered statistically significant.

Results

The baseline demographic characteristics of cases and controls were comparable as shown in table 1. The mean age of the patients were 43.45 years with 56.67% residing in rural areas and 43.33% in urban areas**.** Majority of them were literate Hindus. Age of menarche was less than 15 years in 71% of the total study patients with 88.67% multipara. Body mass index was normal to overweight in majority of the study population with only 5.67 % having a family history of uterine fibroids. The use of OCP was seen only in 17 cases and 7 controls.

The clinical symptoms included prolonged/HMB (74.67%), dysmenorrhea (44.00%), pelvic/abdominal pain (38.00%), anaemia required blood transfusion (31.33%), urinary complaints (20.67%), lump in abdomen (10.67%), dyspareunia (5.33%), infertility (5.33%), and RPL (2%).

Table 1:-Comparison of demographic characteristic between case and control

Demographic characteristics	Case (n=150)	Control (n=150)	Total	P value
Age(years)	43.4 ± 5.28	43.5 ± 5.15	43.45 ± 5.21	0.868†
Area of residence				
Rural	84 (56%)	86 (57.33%)	170(56.67%)	0.816*
Urban	66 (44%)	64 (42.67%)	130(43.33%)	
Education				
Illiterate	25 (16.67%)	28 (18.67%)	53 (17.67%)	0.65*
Literate	125(83.33%)	122(81.33%)	247(82.33%)	
Religion				
Hindu	93 (62%)	97 (64.67%)	190(63.33%)	0.632*
Muslim	57 (38%)	53 (35.33%)	110(36.67%)	
Occupation				
Employed	58 (38.67%)	58 (38.67%)	116(38.67%)	1*
Unemployed	92 (61.33%)	92 (61.33%)	184(61.33%)	
Age of menarche (years)				
<15 years	104(69.33%)	109(72.67%)	213 (71%)	0.525*
>15 years	46 (30.67%)	41 (27.33%)	87 (29%)	
Parity				
Multi	130(86.67%)	136(90.67%)	266(88.67%)	0.274*
Nulliparous	20 (13.33%)	14 (9.33%)	34 (11.33%)	
Body mass index(kg/m²)				
Underweight	12 (8%)	9 (6%)	21 (7%)	
Normal	41 (27.33%)	60 (40%)	101(33.67%)	0.098*
Overweight	52 (34.67%)	49 (32.67%)	101(33.67%)	
Obese	45 (30%)	32 (21.33%)	77 (25.67%)	
Family history	8 (5.33%)	9 (6%)	17 (5.67%)	0.803*
Use of OCP	17 (11.33%)	7 (4.67%)	24 (8%)	0.033*

*- Chi square test, †- Independent t test

Mean ± SD of vitamin D (ng/mL) in control was 31.98 ± 5.33 which was significantly higher as compared to case (16.32 ± 8.58) (p value < 0.0001). Overall vitamin D levels were normal in 75.33% controls and only 12.67% cases. There was a significantly higher vitamin D deficiency in cases as compared to controls (70% vs 4%, p<0.0001) as shown in figure 1.

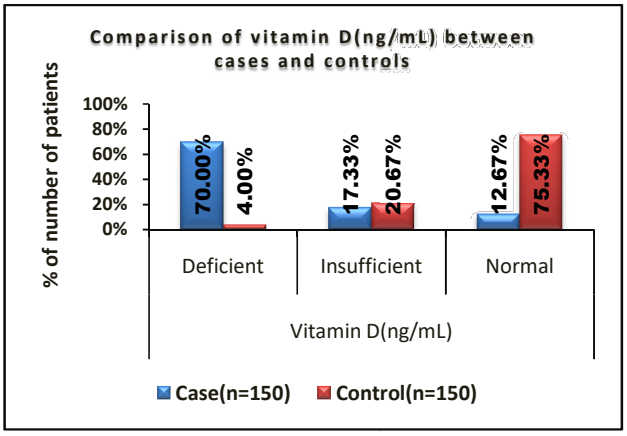

Figure 1: Comparison of vitamin D (ng/mL) between cases and controls

Table 2: Comparison of vitamin D (ng/mL) between age groups and body mass index

Parameters	Vitamin D (ng/mL)	P value
Age (years)		
30-35(n=12)	21.99 ± 7.97	
36-40(n=28)	16.09 ± 8.82	
41-45(n=49)	14.99 ± 7.9	0.015‡
46-50(n=55)	15.51 ± 8.37	
51-55(n=6)	24.27 ± 10.1	
Body mass index (kg/m²)		
Underweight(n=12)	15.65 ± 12.04	
Normal (n=41)	18.31 ± 10.45	0.225‡
Overweight(n=52)	14.62 ± 6.95	
Obese(n=45)	16.64 ± 7.11	

‡ - ANOVA

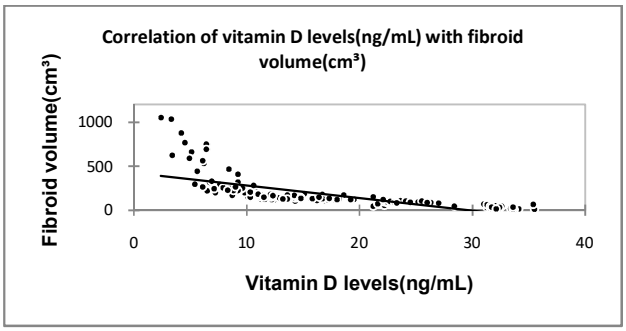

Figure 2: Correlation of vitamin D levels with fibroid volume in cases

Table 3: Association of presenting complaints with vitamin D (ng/mL) in cases

Presenting complaints	Deficient (n=105)	Insufficient (n=26)	Normal (n=19)	Total	P value
Prolonged/HMB					
No	27 (71.05%)	5 (13.16%)	6 (15.79%)	38 (100%)	0.634*
Yes	78 (69.64%)	21 (18.75%)	13 (11.61%)	112 (100%)	
Dysmenorrhea					
No	62 (73.81%)	11 (13.10%)	11 (13.10%)	84 (100%)	0.301*
Yes	43 (65.15%)	15 (22.73%)	8 (12.12%)	66 (100%)	
Pelvic/abdominal pain					
No	62 (66.67%)	19 (20.43%)	12 (12.90%)	93 (100%)	0.416*
Yes	43 (75.44%)	7 (12.28%)	7 (12.28%)	57 (100%)	
Urinary complaints					
No	80 (67.23%)	21 (17.65%)	18 (15.13%)	119 (100%)	0.181*
Yes	25 (80.65%)	5 (16.13%)	1 (3.23%)	31 (100%)	
Dyspareunia					
No	97 (68.31%)	26 (18.31%)	19 (13.38%)	142 (100%)	0.359§
Yes	8 (100%)	0 (0%)	0 (0%)	8 (100%)	
Lump in abdomen					
No	89 (66.42%)	26 (19.40%)	19 (14.18%)	134 (100%)	0.019§
Yes	16 (100%)	0 (0%)	0 (0%)	16 (100%)	
Anaemia required blood transfusion					
No	62 (60.19%)	23 (22.33%)	18 (17.48%)	103 (100%)	0.0005*
Yes	43 (91.49%)	3 (6.38%)	1 (2.13%)	47 (100%)	
RPL					
No	105 (71.43%)	24 (16.33%)	18 (12.24%)	147 (100%)	0.026§
Yes	0 (0%)	2 (66.67%)	1 (33.33%)	3 (100%)	
Infertility					
No	101 (71.13%)	24 (16.90%)	17 (11.97%)	142 (100%)	0.254§
Yes	4 (50%)	2 (25%)	2 (25%)	8 (100%)	

*- Chi square test, § - Fisher Exact test

Mean value of fibroid volume (cm³) of study subjects was 190.8 ± 183 with median (IQR) of 136.9(102.8-207.15). A significant negative correlation was seen between vitamin D levels (ng/mL) with fibroid volume (cm³) with correlation coefficient of -0.674, p<0.0001 (figure 2).

Age showed a significant association with vitamin D levels. The age category of 41 to 45 years had the lowest vitamin D levels whereas the age groups of 30 to 35 and 51 to 55 years had normal vitamin D levels (p = 0.015). Body mass index showed no significant association with vitamin D levels (p= 0.225) (table 2).

Among the clinical complaints, the presence of lump in abdomen and anaemia requiring blood transfusion was significantly associated with vitamin D deficiency (table 3).

On performing multivariate logistic regression, vitamin D was independent significant risk factor of uterine fibroid with adjusted odds ratio of 89.109 for deficient vitamin D and 4.810 for insufficient vitamin D taking normal vitamin D as reference. So it can be concluded that patients with low vitamin D had significantly higher chances of uterine fibroid. It is shown in table 4.

Table 4: Multivariate logistic regression to find out independent risk factors of uterine fibroid.

Variables	Beta coefficient	Standard error	P value	Odds ratio	Odds ratio lower bound (95%)	Odds ratio upper bound (95%)
Body mass index(kg/m²)	0.023	0.04	0.577	1.023	0.945	1.107
OCP use	-0.14	0.622	0.822	0.869	0.257	2.941
Vitamin D(ng/mL)						
Normal				1		
Deficient	4.49	0.476	< 0.0001	89.109	35.037	226.631
Insufficient	1.571	0.371	< 0.0001	4.81	2.323	9.957

Discussion

We found that low vitamin D was an independent significant risk factor of uterine fibroid with a significant correlation with fibroid volume. Our findings were similar to study by Singh et al [6], who reported that mean vitamin D_3 was significantly lower in cases than controls (10.81 ± 6.18 vs. 22.91 ± 16.18, p < 0.0001) where 62.5% of

cases were found to be severely deficient (vitamin $D_3 < 10$ ng/mL) as compared to 26.39% of controls ($p < 0.0001$) and only 2.77% of cases had sufficient vitamin D level as compared to 23.61% of controls ($p = 0.0002$). The OR of occurrence of fibroid with serum vitamin D_3 level of < 10 ng/dl compared to that of level > 10 ng/dl was 4.64 (95% CI, 2.28–9.44) ($p = 0.0001$). This finding connotes a possible inverse correlation between serum vitamin D_3 and uterine fibroid and proposes a cut off of 10 ng/dL for predicting the occurrence of fibroids.

Even in the study by Srivastava et al [7], the serum levels of vitamin D_3 were significantly higher in the women with leiomyomas than in the controls. In cases, women in the insufficiency range and the deficiency were more than controls, whereas in controls, vitamin D was more in normal range. The crude odds ratio (OR) for the presence of leiomyomas on women with serum levels of vitamin D_3 was 20.4 ($p < 0.001$) which was significant but lower than the present study. In addition, there was a statistically significant difference with the mean uterine fibroid size; being greatest in those having a vitamin D_3 deficiency, followed by those having a vitamin D_3 insufficiency and a vitamin D_3 sufficiency; findings similar to the present study.

Our findings were also in line with the results of Paffoni et al [8] as there were lower mean serum levels of 25-hydroxyvitamin D_3 in women with fibroid than controls (18.0 ± 7.7 vs. 20.8 ± 11.1 ng/mL, $p = 0.010$) with a crude odds ratio (OR) for the presence of fibroids in women with serum levels of 25-hydroxyvitamin D_3 less than 10 ng/mL compared with those with 25-hydroxyvitamin $D_3 > 10$ ng/mL as 2.2 (95% CI 1.1–4.3) ($p = 0.022$).

Less vitamin D among cases as compared to controls have also been shown by other studies such as Sabry et al [9] (19.7 ± 11.8 vs. 22.3 ± 6.5 ng/mL, $p = .01$); and Oluwakemi et al, [10] (13.5ng /ml vs 52.1 ng/ml) ($p = < 0.001$). Oluwakemi et al [10], also evidenced the inverse correlation between vitamin D and fibroid volume (Spearman Rho= - 0.113, $p = 0.189$) similar to our study.

The reason for plasma vitamin D levels in women with uterine fibroids being significantly lower than in those without fibroids could be because of the intricate link between the extracellular matrix (ECM) proteins, vitamin D and fibroids. It has been seen that vitamin D is an inhibitor to the loop between TGF -ß3 mediated ECM production, fibrosis and over production in human leiomyomas of collagen type 1, fibronectin, laminin and proteoglycans. In cases with deficient vitamin D, this loop gets a positive feedback without inhibition causing increased production of ECM proteins and thereby proliferation of muscles and fibrosis and finally development of fibroids.

In our study, the mean values of vitamin D showed an inverted U shaped curve with high values at extreme of age groups included in the study and low values in the middle age group. However vitamin D deficiency was comparable among the different study age groups of the cases ($p>0.05$), annulling any effect of age of vitamin D deficiency and fibroids.

Overall, the study demographics of the population showed that the cases were comparable to controls in terms of residence, education, religion, occupation, age of menarche and parity. This ensured that these factors did not have any effect on the difference in the vitamin D levels and the occurrence of uterine fibroids among the study groups. The findings were in line with some of the other comparative studies which showed that the baseline demographic characteristics were comparable ($p>0.05$) [8, 9]. In the study by Singh et al [6], residence, parity, and age at menarche were comparable where in both the groups most of the women were from urban area (70.83% vs. 68.06%, P=0.856); were para 2 (38.89% vs. 43.06%, p=0.93), with age at menarche (12.40 ± 1.03 vs. 12.64 ± 1.03, p=0.164). Oluwakemi et al [10], reported that there was no significant difference in the age at menarche, and parity between the study and control group ($p >0.05$).

Deficiency of vitamin D in India is in epidemic proportion and females suffer the most. Dietary deficiency is the primary etiology. In addition, the incidence of uterine fibroid in reproductive age group was reported 37% in a study conducted in South India.[11] This number in all probabilities might be higher as many more are undetected. So implication of vitamin D deficiency in uterine fibroid will bring in a whole new therapeutic aspect and it will impart a huge impact in the outcome [6].

Limitations of the study -

- The smoking status of the patients was not evaluated since it may affect the occurrence of fibroids.
- The level of sun exposure was not assessed.
- There was lack of detailed assessment of the dietary habits of the patients.
- Our study was conducted in a setting which caters to patients belonging primarily to the lower or middle socio-economic strata and the data primarily reflects the situation in this cohort.

- Being a single centre hospital based study, its results cannot be extrapolated to study the prevalence of vitamin D deficiency and its association with uterine fibroids in the general population.

Conclusion

Low vitamin D was an independent significant risk factor of uterine fibroid with a significant correlation with fibroid volume. Findings of the study suggest that patients with low vitamin D had significantly higher chances of uterine fibroid. Our study showed a definite indirect association of vitamin D deficiency and uterine fibroid in this part of India. This opens up an interesting facet of dietary intake or supplementation of Vitamin D in prevention as well as treatment of uterine fibroid in women of child bearing age.

Conflict of interest: None. **Disclaimer:** Nil.

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